



PHARMACOGNOSTICAL STANDARDIZATION AND PHYTOCHEMICAL PROFILE OF RHIZOMES OF *Drynaria quercifolia* (Linn) J. Smith

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ABSTRACT

Drynaria quercifolia (Linn.) J. Smith (Family: Polypodiaceae) commonly known as Oak leaf fern. Rhizome of this plant traditionally used in the treatment of hectic fever, dyspepsia and cough, antihelminthic, anti arthritic etc. Micromorphology and physicochemical analysis of the rhizomes of *D. quercifolia* to be performed as per WHO and Pharmacopoeial methods. Rhizomes are 2cm or thicker, fleshy, creeping nature and densely clothed with reddish brown soft scales. Microscopic evaluation of rhizome consists of a thin wavy epidermal layer. Epidermis made by parenchymatous tissues which are polyhedral, thin walled and compact. Single layer of thick walled endodermis and layers of pericycle were the cells are rectangular and thin walled. Xylem consists of wide, angular, thick walled metaxylem elements and 4 (or) 5 exarches protoxylem elements. The metaxylem elements are upto 30µm in diameter. Phloem elements are in one or two layers and thick walled. Powder microscopy showed the presence of scales, covering trichome, parenchyma, xylem elements and lignified sclerenchyma bundle. Preliminary phytochemical screening of appropriate solvent extracts showed the presence of sterols, tannins, proteins and amino acids, flavonoids, terpenoids, saponin, carbohydrates and absence of alkaloids, glycosides and volatile and fixed oil. Microscopic analysis and other parameters were informative and provide valuable information in the identification, standardization of *D. quercifolia* rhizome.

KEYWORDS: *Drynaria quercifolia*, Polypodiaceae, Rhizome, Microscopical evaluation.

1. INTRODUCTION

Drynaria quercifolia (L.) J. Smith (Family: Polypodiaceae) is epiphytic (growing on trees) or epipetric (growing on rocks) medicinal fern.^[1] It is distributed widely in the evergreen forests of the Western Ghats of Kerala, and locally called Marappan kilangu or Attukal kilangu^[2]. *Drynaria quercifolia* (Oak leaf fern) is a large species of fern with deeply pinnatifid foliage fronds. It is native to India, Southeast Asia, Malaysia, Indonesia, the Philippines, New Guinea, and Australia.^[3] Traditionally, the soup prepared from the rhizome of *D. quercifolia* is very popular among tribes of Eastern Ghats, Tamil Nadu and it is used to get relief from rheumatic complaints.^[4] The rhizome is reported to be used by tribal communities of Tamil Nadu and Kerala to cure various diseases like phthisis, dyspepsia, cough, typhoid, jaundice, fever, headache and skin disease.^[5,6] Rhizome of this plant has been reported as antioxidant^[7-9], analgesic^[10], anti fertility^[11,12], antimicrobial^[13-15], anthelmintic^[16], antipyretic^[17,18], anti-inflammatory^[19],

antidiabetic and hypolipidemic^[20], antiarthritic^[21, 22], anti urolithiatic^[23], cytotoxic^[24] and mosquito repellent^[25]

Taxonomy^[26]

Kingdom	- Plantae
Division	- Pteridophyta
Class	- Polypodiopsida/Pteridopsida
Order	- Polypodiales
(Unranked)	- Eupolypods
Family	- Polypodiaceae
Subfamily	- Polypodioideae
Genus	- <i>Drynaria</i> (Bory) J. Sm
Species	- <i>quercifolia</i>

Vernacular Names^[27,28]

English	- Oak Leaf Fern
Tamil	- Mudavattukal
Sanskrit	- Aswakatri
Malayalam	- Matilpanna, Pannakizhangu, Pannikizangu
Kannada	- Hanumana hashta, Hanumana paada

Hindi - Asvakatri, Katikapan, Basingh

Various phytoconstituents has been reported in this plant such as 3, 4-dihydroxybenzoic acid friedelin, epifriedelinol, β -amyrin, β -sitosterol and β -sitosterol 3- β -D-glucopyranoside.^[29]

As mentioned earlier several reports have been published regarding chemical constituents and different biological activities *in-vitro* and *in-vivo*. An investigation to explore its pharmacognostic examination is inevitable. The present work was undertaken with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant *Drynaria quercifolia* rhizome to treat various diseases and disorders.

2. MATERIALS AND METHODS

2.1: Chemicals

Formalin, acetic acid, ethyl alcohol, chloral hydrate, toluidine blue, phloroglucinol, glycerin, hydrochloric acid and all other chemicals used in this study were of analytical grade.

2.2: Collection of Specimens and authentication

The rhizome of *D. quercifolia* were collected from Kolli hills, Namakkal, Tamil Nadu and was authenticated by Dr. P. Jayaraman, Director of Plant Anatomy Research Institute, Tambaram, Chennai, Tamil Nadu, India (PARC/2014/2226).

2.3: Macroscopic analysis

Macroscopic observation of the plant was done. The shape, size, surface characters, texture, colour, odour, taste etc was noted.^[30]

2.4: Microscopic analysis

The seeds were fixed in FAA (Formalin - 5 ml + acetic acid - 5 ml + 70% ethyl alcohol - 90 ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary-butyl alcohol (TBA). Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60 C), until TBA solution attained super saturation. The specimens were cast into paraffin blocks^[31].

Sectioning

The paraffin embedded specimens were sectioned with the help of rotary microtome. The thickness of the sections was 10-12 μ m. After dewaxing the sections were stained with toluidine blue. Since toluidine blue is a polychromatic stain, the staining results were remarkably good and some cytochemical reactions were also obtained. The dye rendered pink color to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc.^[32]

Photomicrographs

Photographs of different magnifications were taken with Nikon lab-photo 2 microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light

was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background.

2.5: Powder microscopy

Coarse powder of the leaf was used to study the microscopical characters of the leaf powder^[33, 34].

2.6: Physicochemical analysis

Total ash, acid insoluble ash, water soluble ash, loss on drying and extractive values were determined^[35, 36].

2.7: Preliminary phytochemical screening

Preliminary phytochemical screening was carried out to find out the presence of various phytoconstituents using standard procedure^[37, 38].

3. RESULTS

3.1 Macroscopy

Drynaria quercifolia (L.) J. Smith (Polypodiaceae), commonly known as Oak leaf fern. It is an epiphytic or epipetric medicinal fern with deeply pinnatifid foliage fronds. The nest fronds resemble the leaves of oaks, hence the common name. The sori are either scattered or arranged in two regular rows in between the secondary veins (Figure 1). Rhizomes are 2cm or thicker, fleshy, creeping nature and densely clothed with reddish brown soft scales (Figure 2).



Figure 1: Habit and Habitat of *D. quercifolia*



Figure 2: Rhizomes of *D. quercifolia*

3.2 Microscopy of the Rhizome

The rhizome is thick and woody densely clothed by dark epidermal scales. In cross sectional view, the rhizome consists of a thin wavy epidermal layer.

The surface of the rhizome has wide semi circular ridges and narrow deep furrows from where the scales arise (**Fig 3 & 4**).

The rhizome consists of homogeneous parenchymatous tissue which are polyhedral, thin walled and compact. Scattered in the ground tissue there are several independent steles of different shape and size.

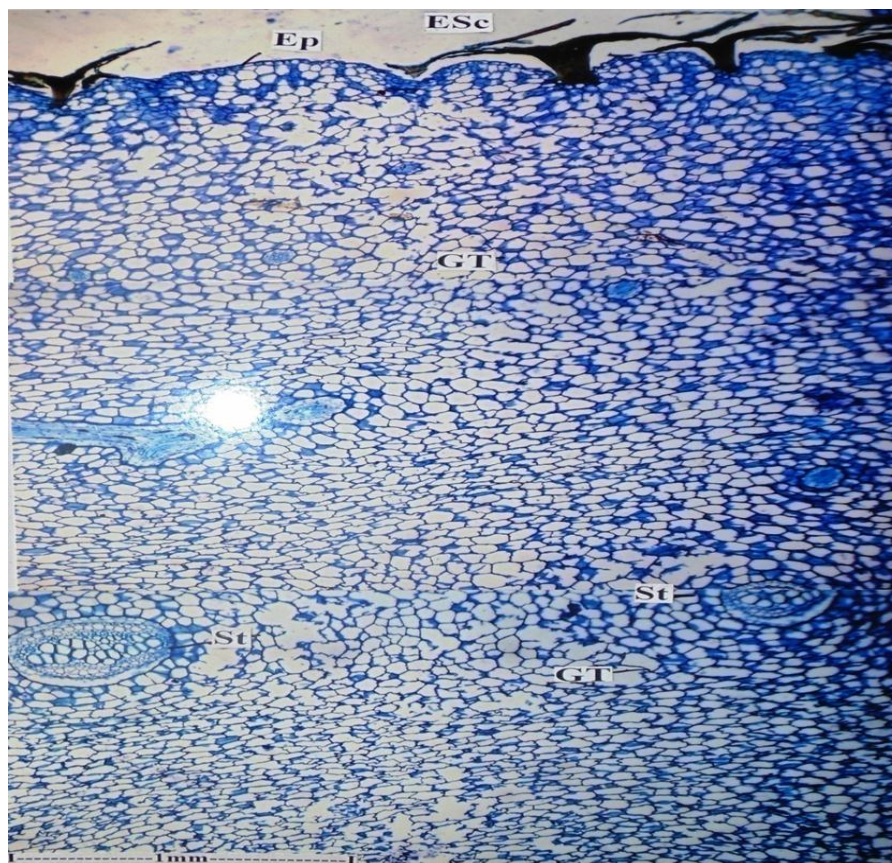
The steles are diffuse in distribution. The steles towards the periphery of the rhizome are smaller and those which are interior are large (**Fig 3, 5, 7 & 8**).

The smaller and larger steles are basically similar in structure. The smaller steles are circular measuring about 70µm in diameter. It consists of a single layer of thick walled endodermis and layers of pericycle where the cells are rectangular and thin walled. Inner to the pericycle is

another layer of thin walled cells which are similar to the pericycle.

The central part of the stele includes xylem and phloem. The xylem is in the centers which are surrounded by phloem. Xylem consists of wide, angular, thick walled metaxylem elements and 4 (or) 5 exarches protoxylem elements. The meta xylem elements are up to 30µm in diameter. Phloem elements are in one or two layers and the cells are small and thick walled (**Fig 7**).

Some of the stele which are towards the centre are elongated and cylindrical (or) elliptical (**Fig 6 & 8**). The elongated steles have endodermal layer which consists of radially oblong thick walled cells. Inner to the endodermis is a narrow circle of pericycle and another inner layer of cells resembling the pericycle. The stele has central, elongated segment of xylem which are surrounded all around by phloem elements. The metaxylem elements are in the centre and protoxylem elements are along the periphery of the xylem segment. Outer portion of the xylem segment is occupied by phloem elements and wide angular thin walled parenchyma cells (**Fig 6 & 8**).



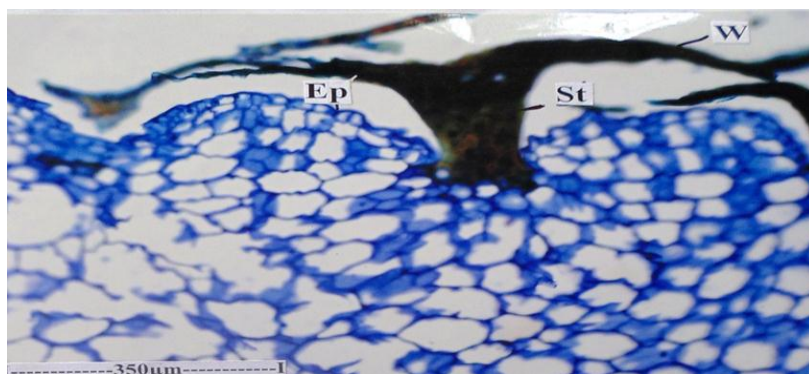
(Ep-Epidermis, ESc-Epidermal scales, GT-Ground tissue, St-Stele)

Figure 3: T.S of Rhizomes of *D. quercifolia*

Scales on the Rhizome

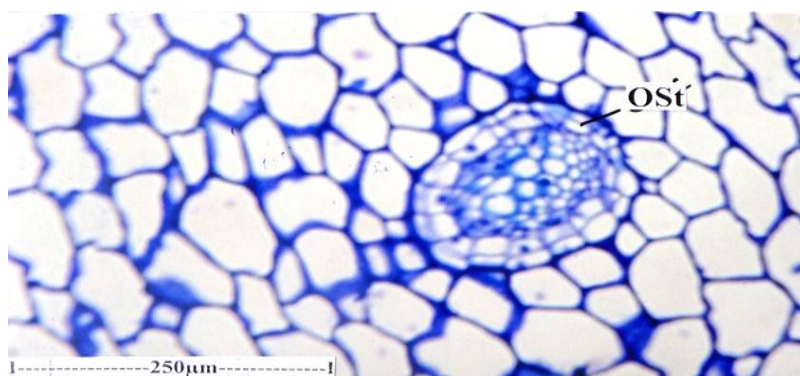
The scales thickly cover the surface of the rhizome. The scales are peltate. They have a central (or) exocentric thick cylindrical stalk at the top of which the scale

spreads over the surface of the rhizome. These wings like upper part of the rhizome are thick in the centre and gradually become thin and membranous towards the periphery. The scales are darkly coloured (**Fig 4**).



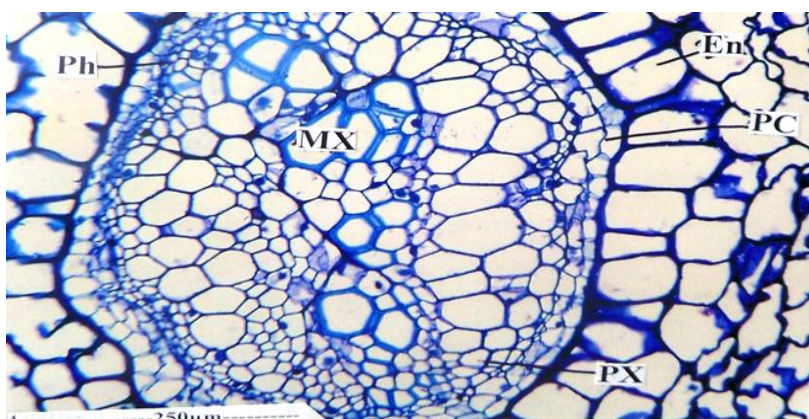
(Ep-Epidermis, St- Stalk, W- Wing)

Fig 4: T.S of rhizome outer part showing the scales and thick stalk attached to the outer part of the rhizome



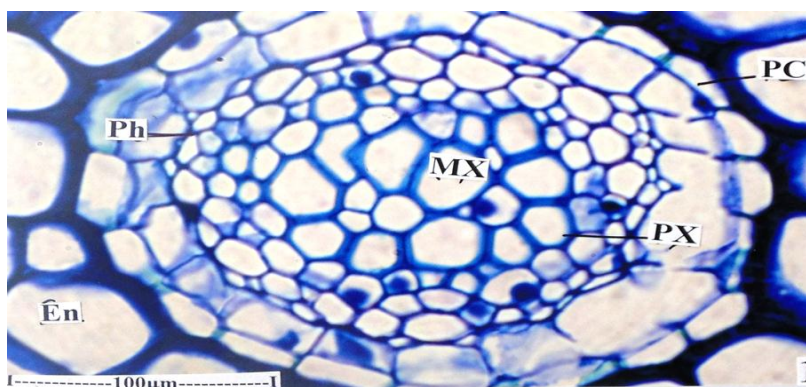
(Ost-Outer Stele)

Fig 5: T.S of rhizome showing outer smaller vascular bundle



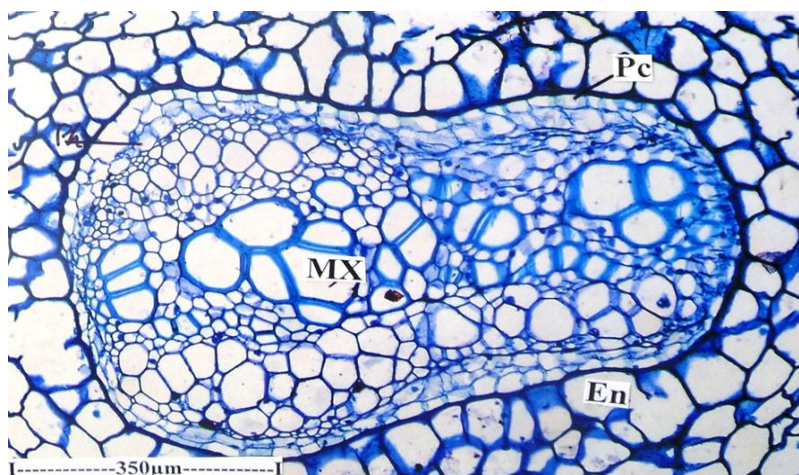
(Mx- Metaxylem, Ph- Phloem, Px- Protoxylem, En- Endodermis, Pc-Pericycle)

Fig 6: Central part of the rhizome showing larger stele



(En- Endodermis, Mx- Metaxylem, Pc-Pericycle, Ph- Phloem, Px- Protoxylem)

Fig 7: Circular type of stele in sectional view



(En- Endodermis, Mx- Metaxylem, Pc- Pericycle, Ph- Phloem)

Fig 8: Cylindrical types of stele in sectional view

3.3: Powder Microscopy

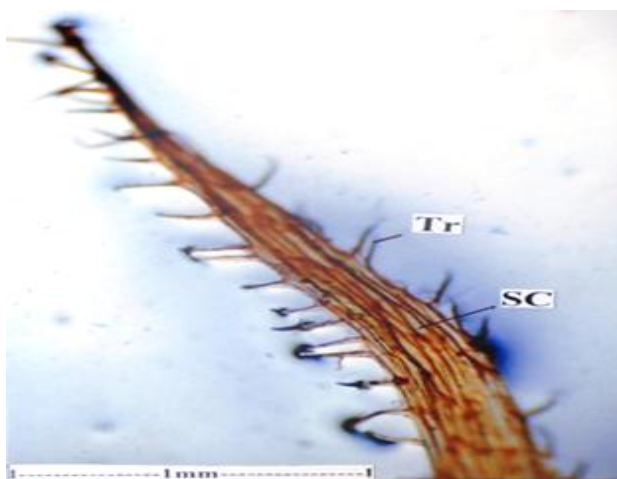
Organoleptic characters

Nature	-	Coarse powder
Colour	-	Brown
Odour	-	Agreeable odour
Taste	-	Slightly Bitter

The powder microscopy of the seeds shows the following characters:

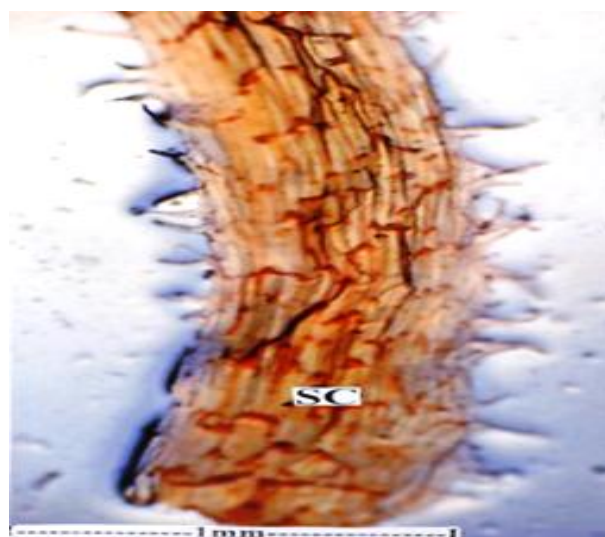
Scales

The scales are of 2 types. Most of the scales are slightly broad at the base and gradually become tapering towards the tip. The basal part of the scale is 700µm wide. The sub terminal part of the scale is about 150µm broad. The cells of the trichome are thick walled vertically elongated and arranged parallel to each other compactly (**Fig 9 & 10**). The two margins of the scale bear long narrowly triangular thin lateral trichomes. Occasionally the trichome produces terminal branches. Some of the scales possess exocentric stalk with the lateral wing extended along the lateral sides of the stalk. The cells of the scale radiate from the central stalk and remain compact in thin plate (**Fig 11 & 12**).



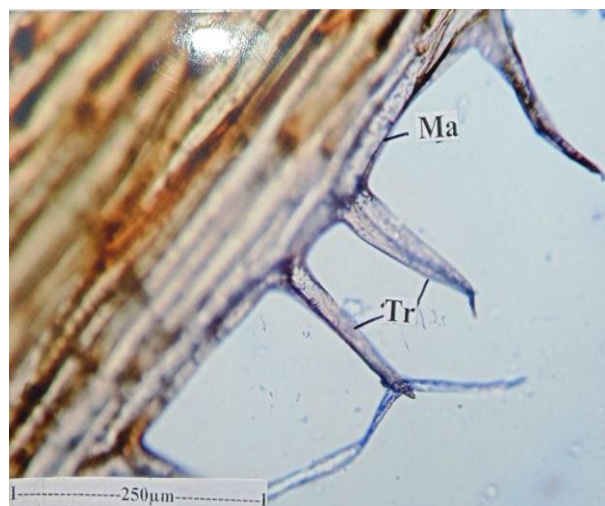
(Tr- Trichome, Sc- Scales)

Fig 9: Scale – Terminal part



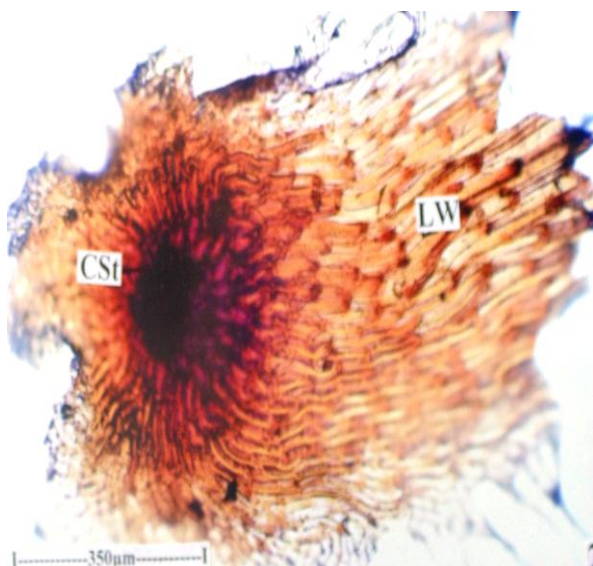
(Sc- Scales)

Fig 10: Scale – Basal part



(Ma- Margin, Tr- Trichome)

Fig 11: Marginal part of the scale showing the trichomes

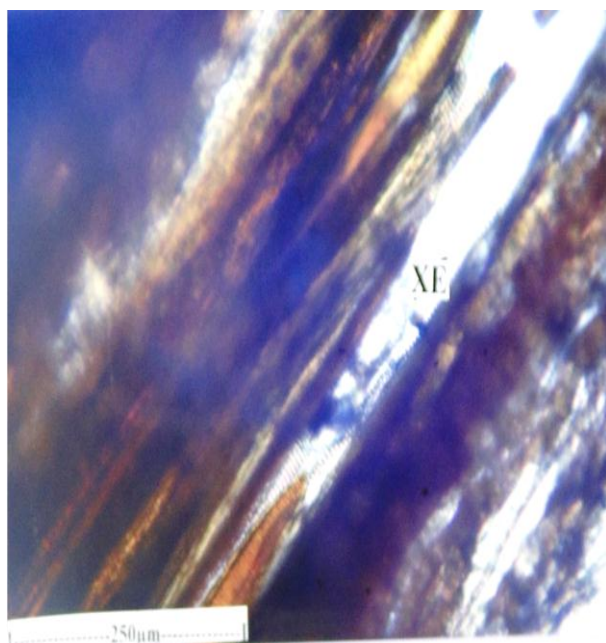


(Cst- Central Stalk, Lw- Lateral Wing)

Fig 12: Exocentric peltate scale

Xylem Elements

The xylem elements are seen in the form of thick bundle. The bundle includes spiral, scalariform and reticulate tracheids. The tracheids are narrow and much longer and thick walled (**Fig 13**).



(XE- Xylem Element)

Fig 13: Xylem elements of the rhizome

Sclerenchyma

Occasionally seen in the powder and spindle shaped dark brown flat bodies comprising highly thick walled compact, sclerenchyma bodies appear bright under the polarized light indicating that the cells are lignified (**Fig 14 & 15**). The sclerenchyma bodies are 250µm long and 130µm wide.



(Scl- Sclerenchyma bundle).

Fig 14: Elliptical bundle of Sclerenchyma which is the basal part of the scale

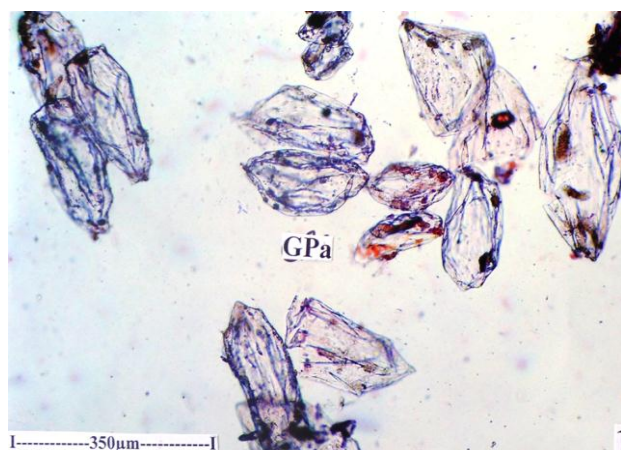


(Scl- Sclerenchyma bundle)

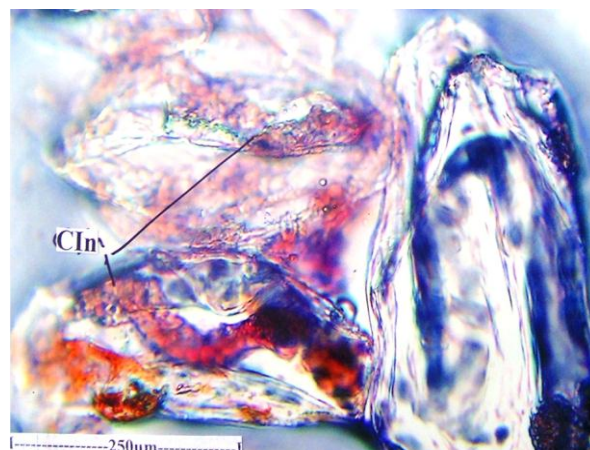
Fig 15: Elliptical bundle of Sclerenchyma under polarized light

Parenchyma

The ground parenchyma cells are seen separated and isolated bodies. The cells are long and elliptical, thin walled and dilated. They are 220µm long and 100µm thick. The parenchyma cells have darkly stained spherical (or) cylindrical bodies inside the cells (**Figure 16 & 17**).



(GPa- Ground parenchyma)

Figure 16: Isolated parenchyma cells from the ground tissue

(CIn- Cell Inclusion)

Figure 17: Cell inclusions in the parenchyma cells**3.4: Physical parameter****3.4.1: Ash Value and LOD of Rhizomes of *Drynaria quercifolia* (L.) J.Smith**

Range	Total Ash (%)w/w	Acid Insoluble ash (%)w/w	Water soluble Ash (%)w/w	LOD
Minimum	7.52	2.22	5.12	40
Average	7.88	3.54	5.38	43.5
Maximum	8.25	5.31	5.70	47

3.4.2: Extractive Values of Rhizomes of *Drynaria quercifolia* (L.) J.Smith

Solvent	Method of Extraction	Extractive value (% w/w)
Petroleum ether	Continues hot percolations (Soxhlet apparatus)	6.10
Chloroform		2.12
Ethyl acetate		3.85
Ethanol		7.92
Water		16.34

3.5: Preliminary Phytochemical Screening of Rhizomes of *Drynaria quercifolia* (L.) J. Smith**Preliminary Phytochemical Screening of Different Solvent Extracts**

Tests	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
Alkaloids					
Mayers Reagent	-	-	-	-	-
Dragendorffs reagent	-	-	-	-	-
Hagers reagent	-	-	-	-	-
Wagners reagent	-	-	-	-	-
Carbohydrates					
Molishch's Test	-	-	+	+	+
Fehlings Test	-	-	+	+	+
Benedicts Test	-	-	+	+	+
Glycosides					
General Test	-	-	-	-	-
Anthraquinone	-	-	-	-	-
Cardiac	-	-	-	-	-
Cyanogenetic	-	-	-	-	-
Coumarin	-	-	-	-	-
Phytosterols					
Salkowski Test	+	+	+	+	-
Liebermann Burchard Test	+	+	+	+	-

Saponins	-	-	+	+	+
Tannins	-	-	+	+	+
Proteins & Free Amino Acid					
Millons test	-	-	+	+	+
Biuret test	-	-	+	+	+
Gums & Mucilage	-	-	-	-	-
Flavonoids					
Shinoda test	-	-	+	+	+
Alkaline Reagent test	-	-	+	+	+
Terpenoids	+	+	+	+	-
Fixed Oil	-	-	-	-	-

4. DISCUSSION

Organoleptic evaluation of a crude drug is mainly for qualitative evaluation based on the observation of morphological and sensory profile.^[39] Hence we have undertaken this study to serve as a tool for developing standards for identification, quality and purity of rhizomes of *Drynaria quercifolia*.

Adulteration and misidentification of crude drugs can cause serious health problems to consumers and legal problems for the pharmaceutical industries. The observation of cellular level morphology or anatomy is a major aid for the authentication of drugs.^[40] Microscopic evaluation is one of the simplest and cheapest methods for the correct identification of the source of the material.^[41]

Microscopic evaluation of rhizome consists of a thin wavy epidermal layer. Epidermis made by parenchymatous tissues which are polyhedral, thin walled and compact. Single layer of thick walled endodermis and layers of pericycle where the cells are rectangular and thin walled. Xylem consists of wide, angular, thick walled metaxylem elements and 4 (or) 5 exarches protoxylem elements. The metaxylem elements are up to 30µm in diameter. Phloem elements are in one or two layers and thick walled. A Pharmacognostical study on the leaf of *D. quercifolia* was previously published^[42]. No report available on therapeutically important rhizome portion. Powder microscopy showed the presence of scales, covering trichome, parenchyma, xylem elements and lignified sclerenchyma bundle.

The ash values are particularly important to find out the presence or absence of foreign inorganic matter such as metallic salts and or silica (earthy matter).^[43] Acid insoluble ash provides information about non-physiological ash produced due to adherence of inorganic dirt, dust to the crude drug^[44]. Phytochemical evaluation and molecular characterization of plants is an important task in medicinal botany and drug discovery^[45]. Preliminary phytochemical screening of appropriate solvent extracts showed the presence of sterols, tannins, proteins and amino acids, flavonoids, terpenoids, saponin, carbohydrates and absence of alkaloids, glycosides and volatile and fixed oil.

5. CONCLUSION

D. quercifolia has a wide range of phytochemicals which could be useful for treatment of various diseases. Many reports were done on screening of rhizome of *Drynaria quercifolia* both *in-vivo* and *in-vitro* exhibited its potency to cure diseases. No report available on quality assessment of rhizome of *D. quercifolia*. Keeping in this view an attempt was made for standardization of purity and quality of therapeutically valuable rhizome part of *Drynaria quercifolia* (L.) J. Smith.

Conflict of interest statement: We declare that we have no conflict of interest.

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